

Will malaria soon be a thing of the past?the potential of recombinant protein vaccines to control one of the world's most deadly diseases

Created: June 26, 2006

An effective malaria vaccine may well be on the way. These days, vaccines are a normal part of our lives. Most United States children are vaccinated for some form of hepatitis, along with a slew of other diseases including polio, rubella, measles, and mumps. Some of us take these things for granted, but in other parts of the world, vaccines are not so easily obtained.

Malaria, globally one of the most devastating diseases affecting humans, is caused by four species of the *Plasmodium* genus. Of the four species that can cause malaria in humans, *Plasmodium falciparum* is the one that causes most deaths (1). Malaria could be made significantly less deadly by using a cheap, easy-to-produce vaccine.

Traditionally, vaccines have been made from attenuated viruses or bacteria, or by creating a virus-like particle, in the case of some types of flu vaccine(2). Another method for creating vaccines, recently becoming more popular, is to create a recombinant protein. Vaccines made with recombinant proteins offer an advantage over other types of vaccines in that there is no need to handle the actual disease-causing agent, which can be costly and sometimes dangerous. Instead, one or more proteins are expressed and purified, then formulated to be injected into the subject to cause an antibody response against the foreign protein. If, in the future, that person is again exposed to the same protein, it is hoped that his or her immune system will recognize it more quickly as a threat. Recombi-

nant protein vaccines are currently being researched and tested for a variety of diseases, including ricin toxin exposure, pneumococcus infection, and malaria (3-5). There are currently a few different recombinant protein vaccines against *P. falciparum* being tested in clinical trials [<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=malaria.table.2474>], including apical membrane antigen 1 (AMA1) (6).

How did we arrive at this point? As early as 1997, scientists were testing some form of AMA1 for its antibody response against *Plasmodium* species (7). But to use a protein as a vaccine, it must be economically feasible to create large amounts of the protein. The *Plasmodium* genome is highly A+T rich, which makes it hard to express *P. falciparum* proteins in sufficient yields for commercial use in classic expression systems such as *Escherichia coli* and *Pichia pastoris*. One way to augment expression in such species is to recode the gene to match the host's tRNA pool. DNA codons that are rare in the target species are replaced with those that are used more often, while keeping the amino acid sequence unchanged. This raises protein yield because more tRNA molecules exist in the cell for those codons, making protein synthesis easier.

Genes that are recoded, or "synthetic [<http://www.evolvecode.net/codon/sgdb>]" have been used for years to raise yields and reduce costs for many medically and industrially useful proteins, such as insulin (10). For AMA1, *Pichia pastoris* is the most

widely used expression system, because the protein can be expressed in much greater quantities than in the original host organism (8, 9).

To remedy this, some AMA1-derived vaccines, such as AMA1-C1, are mixtures of AMA1 cloned from different strains of *P. falciparum* (9). These combination vaccines are intended to elicit better antibody responses against diverse strains of *P. falciparum* than any one strain-specific AMA1 protein (11).

Manufacturing a cheap, effective vaccine for malaria will depend on many factors. A large-scale method for preparing AMA1-derived vaccines is still far from a reality, and limited human trial data is available (9). There are still three other species of *Plasmodium* that can cause malaria, so effective vaccines must be considered for these, especially because it has been shown that *P. vivax* can replace *P. falciparum* in areas in which the *falciparum* species has been contained (6). Interest has also been shown in certain oligodeoxynucleotide (ODN) molecules that, when added to the vaccine formulation, may strengthen the immune response against AMA1-derived vaccines (11).

Protein vaccines, as compared to other types of vaccines, could potentially be cheap, easy to produce vaccine candidates against malaria, one of the world's deadliest diseases. Promising early research results have been shown, but much research must still be done to make a malaria vaccine a reality. Clinicians and researchers have their work cut out for them, as always. For now, effective treatment is still extremely important in the fight against devastating diseases such as malaria, but in the next few years, that may change.

This Coffee Break was contributed by Tyler Beck, during an internship at the National Center for Biotechnology Information, while on sabbatical from the University of Maryland, Baltimore County.

References

1. Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, Jha P, Mills A, Musgrove P. 21. Conquering Malaria. In: Disease control priorities in developing countries. Washington (DC):IBRD/The World Bank and Oxford University Press; 2006.
2. Galarza JM, Latham T, Cupo A. Virus-like particle (VLP) vaccine conferred complete protection against a lethal influenza virus challenge. *Viral Immunol* 18(1):244–251; 2005. (PubMed)
3. Jomaa M, Terry S, Hale C, Jones C, Dougan G, Brown J. Immunization with the iron uptake ABC transporter PiaA and PiuA prevents respiratory infection with *Streptococcus pneumoniae*. *Vaccine* 24(24):5133–5139; 2006. (PubMed)
4. Vitetta ES, Smallshaw JE, Coleman E, Jafri H, Foster C, Munford R, Schindler J. A pilot clinical trial of a recombinant ricin vaccine in normal humans. *Proc Natl Acad Sci U S A* 103(7):2268–2273; 2006. (PubMed)
5. James S, Miller L. Malaria vaccine development: status report.
6. Matuschewski K. Vaccine development against malaria. *Curr Opin Immunol* 18(4):449–457; 2006. (PubMed)
7. Amante FH, Crewther PE, Anders RF, Good MF. A cryptic T cell epitope on the apical membrane antigen 1 of *Plasmodium chabaudi adami* can prime for an anamnestic antibody response. *J Immunol* 159(11):5535–5544; 1997. (PubMed)
8. Kennedy MC, Wang J, Zhang Y, Miles AP, Chitsaz F, Saul A, Long CA, Miller LH, Stowers AW. In vitro studies with recombinant *Plasmodium falciparum* apical membrane antigen 1 (AMA1): production and activity of an AMA1 vaccine and generation of a multiallelic response. *Infect Immun* 70(12):6948–6960; 2002. (PubMed)(Full text in PMC)
9. Malkin EM, Diemert DJ, McArthur JH, Perreault JR, Miles AP, Giersing BK, Mullen GE, Orcutt A, Muratova O, Awkal M, et al. Phase 1 clinical trial of apical membrane antigen 1: an asexual blood-stage vaccine for *Plasmodium falciparum* malaria. *Infect Immun* 73(6):3677–3685; 2005. (PubMed)(Full text in PMC)

10. Dunn CJ, Plosker GI, Keating GM, McKeage K, Scott LJ. Insulin glargine: an updated review of its use in the management of diabetes mellitus. *Drugs* 63(16):1743–1778; 2003. (PubMed)
11. Mullen GED, Giersing BK, Ajose-Popoola O, Davis HL, Kothe C, Zhou H, Aebig J, Dobrescu G, Saul A, Long CA. Enhancement of functional

antibody responses to AMA1-C1/Alhydrogel, a *Plasmodium falciparum* malaria vaccine, with CpG oligodeoxynucleotide. *Vaccine* 24 (14):2497–2505; 2006. (PubMed)

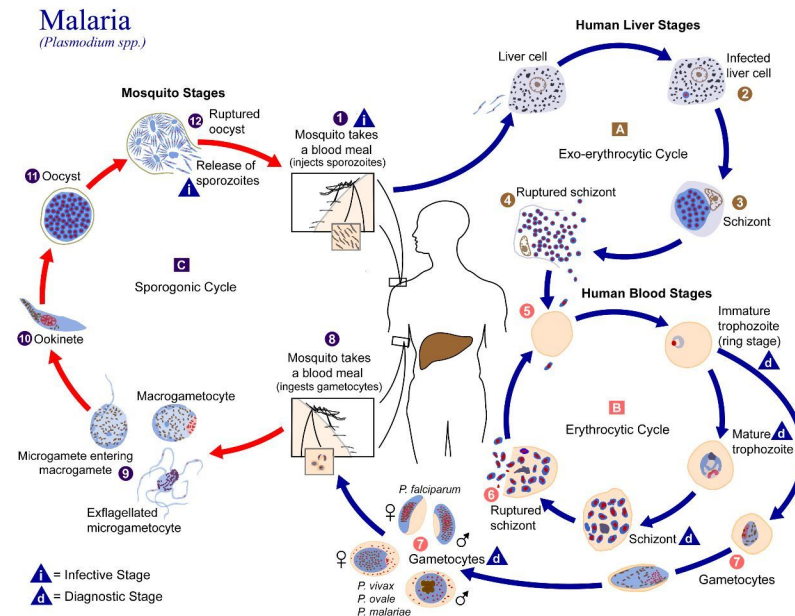


Figure 1: The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host. Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease.

The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinets) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.

Figure and legend reproduced, with permission, from the Centers for Disease Control and Prevention website [http://www.cdc.gov/malaria/biology/life_cycle.htm].

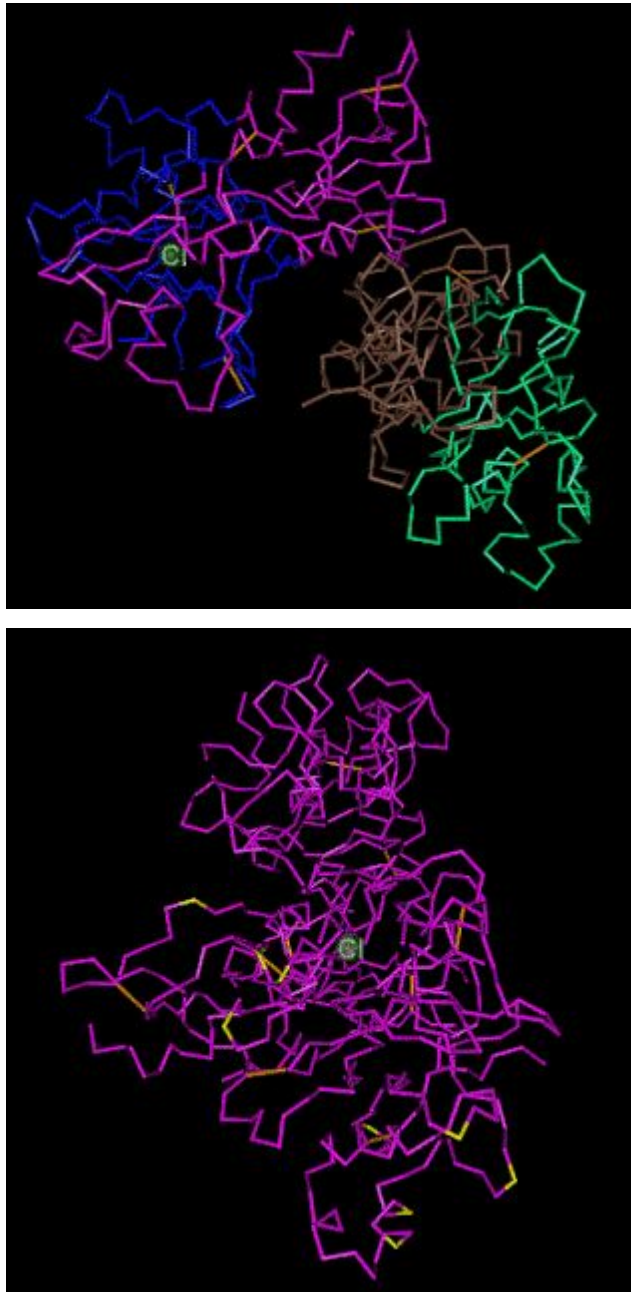


Figure 2: (Top) Three-dimensional view of the AMA1 protein with domains colored differently. You can download the 3-dimensional model [<http://www.ncbi.nlm.nih.gov/books/bookres.fcgi/coffeebrk/ama1.prt>] in [<http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>]. (Bottom) Three-dimensional view of the AMA1 protein with polymorphic residues highlighted.

Link Roundup

NCBI Resources

Natural gene/synthetic gene comparison using BLAST2 [<http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi?PROGRAM=blastn&view=2&ONE=AJ277646&TWO=AF512507>]

Natural protein/synthetic protein comparison using BLAST2 [<http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi?PROGRAM=blastp&view=2&ONE=9931185&TWO=25271701>]

Taxonomy record for *Plasmodium falciparum*

Gene record for *P. falciparum ama1*

External Resources

Clinical trial data from Clinicaltrials.gov [<http://www.clinicaltrials.gov/ct/gui/show/NCT00114010;jsessionid=8EA856FFB153C6AF6D8A3D5D1CFFEB61?order=7>]

The Malaria Vaccine Initiative [www.malariavaccine.org] homepage

NIH News: NIAID and the Malaria Vaccine Initiative/PATH Sign Agreement to Accelerate Malaria Vaccine Research [www3.niaid.nih.gov/news/newsreleases/2000/mvi-path.htm]

See a map of malaria endemicity [www.rbm.who.int/wmr2005/html/map1.htm] from World Malaria Report 2005

AAAS: Malaria and Development in Africa: A Cross-Sectoral Approach [www.aaas.org/international/africa/malaria91/background.html]

The Synthetic Gene Database [www.evolvingcode.net/codon/sgdb/] homepage